

ORIGINAL ARTICLE

From a sequential pattern, temporal adjustments emerge in hummingbird trapliningRunning title: **Hummingbirds trapline on time**Tello-Ramos, Maria Cristina^{1*}, Hurly, T. Andrew² & Healy, Susan D.¹¹ University of St Andrews, Scotland, UK. Harold Mitchell Building² University of Lethbridge, Alberta, Canada.

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Abstract

Animals that feed from resources that are constant in space and that refill may benefit from repeating the order in which they visit locations. This is a behavior known as traplining, a spatial phenomenon. Hummingbirds, like other central-place foragers, use short traplines when moving between several rewarding sites. Here we investigated whether traplining hummingbirds also use relevant temporal information when choosing which flowers to visit. Wild rufous hummingbirds that were allowed to visit three artificial flower patches in which flowers were refilled 20 minutes after they had been depleted, repeated the order in which they visited the three patches. Although they tended to visit the first two patches sooner than 20 minutes, they visited the third patch at approximately 20 minutes intervals. The time between visits to the patches increased across the experiment, suggesting that the birds learned to wait longer before visiting a patch. The birds appeared to couple the sequential pattern of a trapline with temporal regularity, to some degree. This suggests that there is a temporal component to the repeated spatial movements flown by foraging wild hummingbirds.

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Introduction

We might consider a meadow full of flowers a beautiful scene, but for a nectarivore it represents a complex foraging task. Due to their energetically expensive flight and high metabolic rate, hummingbirds need to feed from hundreds of flowers every day to keep a positive energy balance (Kodric-Brown & Brown 1978; Gass & Garrison 1999). To save time and energy, hummingbirds should avoid visiting empty flowers (Healy & Hurly 1995; Hurly 1996). Some flowers are worth revisiting because they refill their nectar supply, making these flowers a renewing resource. As different species of flowering plants refill their nectar at different rates (Castellanos *et al.* 2002, McDade & Weeks 2004) a hummingbird might need to keep track of the nectar content of multiple flowers as well as their refill rate. Foraging then becomes a complex task where animals need to learn and remember multiple types of information such as the spatial location of refilling flowers and the time flowers take to refill.

When hummingbirds first encounter a resource in a new location, visual cues such as the color or shape of a flower may be useful for categorizing the new flowers (Dukas & Waser 1994, Sandlin 2000 a; Sandlin 2000 b). Territorial hummingbirds, however,

remember the locations of profitable flowers and preferentially use the spatial location and not the color of individual flowers they have visited (Healy & Hurly 1995; Healy & Hurly 1998; Henderson *et al.* 2001), even after a single visit (Flores-Abreu *et al.* 2012).

Remembering the spatial location of rewarded flowers allows hummingbirds to return to rewarding flowers and to avoid empty flowers even if those flowers look very similar (i.e., if empty and rewarded flowers have the same color). Nevertheless, because some flowers refill their nectar over different time intervals, a hummingbird cannot know when to revisit a flower using spatial information alone.

Hummingbirds can match the time of their visits to the refill rates of artificial flowers, as shown when hummingbirds trained to feed from artificial flowers that refilled either 10 or 20 minutes after the bird had visited that flower visited the 10-minute flowers significantly sooner than they visited the 20-minute flowers (Henderson *et al.* 2006). Similarly, hummingbirds can learn the time of day at which different patches of flowers are more likely to contain reward (Tello-Ramos *et al.* 2015), known as time-place learning. As hummingbirds feed from hundreds of flowers every day, it would be useful if they could integrate information about a flower's location and its refill rate.

While hummingbirds that live in the tropics might feed from a single flower that produces enough nectar for several visits (McDade & Weeks 2004), hummingbirds in temperate ecosystems typically feed from flowers that produce a small amount of nectar and are patchily distributed. These hummingbirds might be expected to visit all flowers in a patch before moving on to the next one. If they could keep track of the patches they have visited hummingbirds could avoid patches that they have recently emptied. Concomitantly, by repeating the order in which they visit patches of flowers (a foraging behavior known as “traplining”) hummingbirds, as do other nectarivores, eventually start to use routes that reduce the distances they fly between rewarded patches (Tello-Ramos, Hurly & Healy 2015). This foraging behavior is similar to that of the Travelling Salesman problem, in which the salesman is required to visit several locations using the shortest possible route, a problem, which although mathematically complex, animals seem to solve using “simple” foraging rules (Lihoreau *et al.* 2010).

Traplining is taxonomically widespread and seen in bees (Janzen 1971 for the first description of foraging routes of marked euglossine bees; Ohashi & Thomson 2005;

Thomson, Slatkin & Thomson 1997, see also Freeman 1968 for a description of Darwin noting the routes of male “humble bees” around his garden), birds (Gill 1998), primates (Noser & Byrne 2010) and ungulates (Riotte-Lambert, Benhamou & Chamaillé-Jammes, 2016). Although the mechanistic basis of this behavior is not yet clear, traplining is thought to optimize foraging in several ways. First, as animals move systematically through a sequence of locations they avoid revisiting locations they have just depleted (Lemke 1984; Lihoreau *et al.* 2013; Noser & Byrne 2010; Ohashi & Thomson 2009). Second, by traplining, animals might also outcompete intruders since the former have the opportunity to exploit the resource at the right time of day, neither too soon before the resource has refilled, nor too late so that the resource has been depleted by an intruder (Lihoreau *et al.* 2010). For example, at the beginning of each day territorial rufous hummingbirds (*Selasphorus rufus*) appear to visit the patches of flowers at the periphery of their territories and as the day progresses, to systematically “work their way” into the patches closest to their central perch (Paton & Carpenter 1984). In this way, the birds will exploit first those patches within their territories that are most vulnerable to intruders. For a trapline to be efficient, the animal needs to couple the locations of profitable sites together with information on the rate or time at which the reward is likely to have renewed. Visiting locations within a trapline that have not had the time to renew would be a waste of time and energy. Repetitive movements through space would, however, lead to a regular pattern in time (Williams & Thomson 1998) that could be used to match the renewing rate of resources.

Given the number of individual flowers within a hummingbird’s territory, it is possible that the territorial hummingbird faced with a meadow of flowers does not remember the specific time of day at which each flower is rewarded or how long it takes to refill. It is possible however, that by repeating the order in which they visit several locations, hummingbirds could also synchronize their revisits to the refill time of flowers. To test whether wild, free-living hummingbirds can integrate spatial and temporal information when foraging we presented territorial birds with patches of artificial flowers for which we controlled the refill schedule. In previous traplining experiments both with hummingbirds and hymenoptera (Garrison & Gass, 1999; Lihoreau *et al.* 2011; Ohashi & Thomson 2005; Tello-Ramos *et al.* 2015), only the spatial component was manipulated.

But here we used a foraging scenario in which flowers refill after a fixed interval. The aim was to determine first, if hummingbirds foraging from three patches of artificial flowers can match their visits to the refilling interval of the flowers and second, if this matching is accomplished by developing regular sequences between the patches, thus coordinating both spatial and temporal patterns – traplining. In order to determine whether hummingbirds would couple a trapline between several patches to a fixed refill interval, we placed three patches of four artificial flowers within the territory of a male rufous hummingbird. A bird was allowed to visit patches, and flowers within a patch, as he chose, but we refilled each flower only 20 minutes after a bird had visited that flower. In this way, a bird's visit to a flower determined the time at which that flower again contained reward. We provided enough sucrose in the flowers within each patch so that a hummingbird could drink sufficient sucrose for a foraging bout without having to fly to another patch. At this field site, territorial male rufous hummingbirds feed approximately every 10 minutes (Henderson *et al.* 2006; Samuels *et al.* 2014) and they feed almost exclusively from the artificial feeder or artificial flowers we provide (sometimes they will also hawk insects). In this experiment, a bird that fed from the flowers in patch “A” and returned after 10 minutes, would find food only in patches “B” and “C”. Emptying the flowers in, say, patch “B” would leave only the flowers in patch “C” providing reward, at least until 10 minutes after depleting the patch “B” flowers when the flowers in patch “A” would have refilled. If a bird always takes longer to return than 10 minutes, he could feed efficiently by simply alternating his visits between patches “A” and “B” (or any other pair of patches). If, however, he returns in less than five minutes, he needs to have remembered which two of the three patches he has emptied. If a bird could learn the refill schedule of each patch, by repeating the order of visiting the three patches a hummingbird could be sure to avoid visiting empty patches.

Materials and Methods

Subjects and experimental site

The subjects were eight free-living territorial male rufous hummingbirds in the Westcastle Valley in southwestern Alberta, Canada (49°29'N; 114°25'W). Trials were run between 0700 and 1800 hours Mountain Standard Time from June to July 2014. Two weeks prior to

the start of the experiment, we placed 26 hummingbird feeders along the valley. All feeders had a red plastic base and a single yellow plastic flower through which a bird could drink the sucrose solution in the feeder. The feeders contained 20 % sucrose solution (Blem *et al.* 2000), which we refilled whenever the volume got low. Once we identified that a feeder was defended by a single rufous male we trained the hummingbird to feed from the feeder placed inside a mesh trap in order to catch and mark him. We marked birds by painting their breast feathers with nontoxic waterproof ink (Jiffy Eco-marker Ink) and therefore we were able to individually identify birds at each different site. One day after a bird was marked we trained him to feed from an artificial flower (a syringe tip surrounded by a colored cardboard circle of 6 cm in diameter mounted on a 60 cm wooden stick; Fig. 1, Supplementary Material 1). We trained birds by gradually lowering the feeder 20 cm at a time between visits. Eventually we removed the feeder all together and the hummingbird would feed from the artificial flower. Once a bird was feeding regularly from the training flower, we started to pre-train the bird to feed at the different locations.

The University of St Andrews Ethical Committee and the University of Lethbridge Animal Welfare Committee approved all work, which was also conducted under permit from the Alberta Sustainable Resource Development and Environment Canada.

Pre-training

To ensure that hummingbirds would search for sucrose at all the locations at which we would eventually present patches of artificial flowers, we first presented only one flower per patch for a single bout. The single training flower contained 120 μ l of 25% sucrose solution, an amount that is enough for a single bout as these territorial birds usually drink 100 μ l in a bout (Hurly 2003; Karasov *et al.* 1986). Once the bird had fed from that single flower, we removed that flower and placed another flower at the location of the second patch. Once the bird had fed from a flower at all three different patch locations we allowed the bird to feed from the feeder for 20 minutes and then we started the experiment. To prevent the hummingbirds from using the temporal information about their visits to the pre-training flowers at each patch location, we started the experiment only after 20 minutes had elapsed. This allowed the birds to start “counting” the refill time of all flowers from 0 in their first visit during the experiment proper.

Experimental procedure

The three patches of flowers were presented simultaneously and were separated by 3 m to form a regular triangle. The four flowers within each patch were separated by 40 cm forming a square and all flowers were filled with 25 μ l of 25 % sucrose solution (Figure 1). The color of flowers within a patch were all the same but differed among patches and the location of patches of a specific color with respect to the hummingbird's main perch were counter-balanced across birds. To reduce the probability that a bird preferentially visited the patches closest to his preferred perch or the feeder location, two of the patches were placed equidistant to the perch or feeder location. The possible five colors used were: pink, green, blue, purple and orange. We used patches of different colors because even though hummingbirds do not preferentially use color cues to remember rewarded flowers (Lunau *et al.* 2011; Bené 1941; Marshall *et al.* 2012; Hurly & Healy 1996; Tello-Ramos *et al.* 2014), color cues do potentiate the learning of spatial cues and temporal information associated with those rewarded flowers (Samuels *et al.* 2014).

When patches were first presented, all flowers in all patches contained sucrose. Once a hummingbird had visited a flower, that flower was refilled only after a 20-minute interval using a micropipette. If a bird did not drink all the sucrose in a flower the remaining sucrose was emptied and the flower was refilled 20 minutes later. Patches were presented for 200 bouts, a number of bouts that is sufficient to detect repeatability in the order in which hummingbirds feed from several locations (Tello-Ramos *et al.* 2015; birds 1 and 5 only completed 175 and 189 bouts, respectively). Birds took on average $4 \text{ days} \pm 0.59$ (mean \pm S.E., $n = 8$) to complete all the bouts, which was expected since male territorial rufous hummingbirds will feed about every ten minutes throughout the experimental day. A bout was defined as any time the hummingbird came to feed from any of the flowers in the array of patches and ended when the bird left the array area. In order to ensure that the birds did not use the experimenter as a cue to the location of rewarded flowers, every time the experimenter refilled a flower she also mimicked the refilling action at all of the flowers in all three patches.

The bird was free to visit the patches throughout the day. At the end of each experimental day (18:00 hrs) the feeder was returned to its usual location and the flower

patches were removed. The locations of the flowers in each patch were marked with flagging tape so that the next day the flowers would be placed at the exact same location. Similar to previous experimental protocols (Tello-Ramos *et al.* 2015), for each bout the experimenter recorded the order and the time each flower was visited. Since intruders were rare and were not trained to feed from the artificial flowers, we did not record the frequency of intruders or how often the territorial bird revisited a patch after he had chased away an intruder.

Data analyses

Timing of visits to patches

If a bird visited the patches in a regular sequence, one at a time, so that he visited a patch only after he had visited the other two patches, then the inter-visit interval for each patch would approximate 30 minutes. So, in order to test whether that was the case, we used a two-tailed one-sample Wilcoxon signed rank test to compare the actual mean inter-visit interval to each patch for every bird, with the expected 30 minutes. An interval between visits to a specific patch that differed from 30 minutes would suggest that hummingbirds did not feed from one patch at a time in the same order. Alternatively, hummingbirds could have visited the different patches in any sequence if they waited 20 minutes before they revisited a particular patch. Therefore, we also compared, using another two-tailed one-sample Wilcoxon signed rank test, the inter-visit intervals with a 20 minutes interval to determine if birds were more likely to visit a particular patch near the 20 minutes mark. We also tested whether some of the patches were visited more frequently than others using Chi-square tests.

Did the hummingbirds visit the patches in sequence?

If a bird moved sequentially between patches and visited only one patch on each bout, then the number of times he visited a patch as his first choice in a bout should have been equal across the patches. The distribution of the number of first visits to each patch was compared to a random distribution with a Chi-squared goodness of fit test as a proxy of whether hummingbirds were following a repeated order of visitation to the patches.

Additionally, in order to determine whether a bird repeated the order in which he visited the patches, including within or between bouts, we counted the number of times a bird visited a patch having visited another. In this way we constructed a transition matrix with sequence data for each bird as the number of times a bird went from patch “A” to patch “B” or from patch “B” to patch “C” for example, and with the number of times a bird revisited (between bouts) the same patch in succession in the diagonal of the matrix. Then as an independent model, an expected matrix was calculated using the frequency of transitions between patches and a simple probability matrix. A likelihood ratio test was then used to compare the transition matrix to the expected matrix. When the observed and expected matrices were significantly different we also calculated Z-score for each patch transition. Transitions with Z- scores > 1.96 were statistically different at a significant level of 0.05 for this test.

Did the birds avoid revisiting empty flowers?

To estimate whether birds learned to avoid empty flowers, we compared the number of times a bird visited a rewarded flower with the probability of visiting a rewarded flower at chance. The number of possible rewarded flowers changed depending on the interval between visits and on the number of flowers the bird had emptied on the previous visit. The chance level for each bout was set as the number of flowers that were rewarded at each bout divided by the total number of flowers (12). For example, if all the flowers in one patch were empty and all the flowers of the other two patches were rewarded the probability of visiting a rewarded flower was 0.67 (8/12). We then calculated the mean probability of visiting a rewarded flower for each bird and we then used a Binomial test to determine whether a bird was visiting a rewarded flower as its first choice more often than expected by chance. We divided these analyses into bins of 50 bouts (1-50, 51-100, 101-150 and 151-200) so that we would know if experience had an effect on the probability of visiting and empty flower. For this we compared the percentage of visits to a rewarded flower using Wilcoxon matched-pair tests. We considered a significance threshold of $P < 0.01$ throughout.

All analyses we carried out using the statistical software R (version 3.2.3, R Development Core Team 2012).

Results

Timing of visits to the patches

The eight hummingbirds made a total of 1563 bouts to the flower arrays. The average interval at which the birds visited the array of patches at the beginning of the experiment did not differ significantly from that at which they visited the array at the end of the experiment (Wilcoxon matched-pairs test: $W = 16$, $P = 0.79$). During the first 50 bouts birds fed from the array on average every $00:09:10 \pm 0:55$ minutes (mean \pm S.E., $n = 8$) and by the last 50 bouts birds visited the array on average every $00:10:08 \pm 1:32$ minutes (mean \pm S.E., $n = 8$). As expected, hummingbirds visited the array about every 10 minutes.

The visit intervals to all individual patches were all significantly greater against a 10-minute value (two-tailed one-sample Wilcoxon signed rank tests: For all birds $W \geq 1.5$, $P < 0.01$; Figure 2). At the same time, in all cases the inter-visit intervals for each patch were significantly shorter than 30 minutes (tested using a two-tailed one-sample Wilcoxon signed rank test: For all birds $W \geq 1.5$, $P < 0.001$; Figure 2). Rather than waiting 30 minutes to revisit a patch, the birds took on average $17:03 \pm 00:07$ (mean \pm S.E., $n = 8$) minutes to revisit a particular patch (Figure 2). In fact, hummingbirds visited different patches at different intervals. Six of the eight birds visited at least one patch near or after the 20 minutes interval had passed (Birds 1, 2, 3, 4, 5 and 8) and their inter-visit interval to specific patches was not significantly different to 20 minutes (two-tailed one-sample Wilcoxon signed rank test, Bird 1: $W = 1217$, $P = 0.62$; Bird 2: $W = 1030.5$, $P = 0.63$; Bird 3: $W = 1026$, $P = 0.03$; Bird 4: $W = 1348$, $P = 0.05$; Bird 5: $W = 1561$, $P = 0.06$ and Bird 8: $W = 2146.5$, $P = 0.10$). While Bird 1 visited not only one, but two, of the patches when 20 minutes from his last visit to that patch had passed, Birds 6 and 7 visited all three patches significantly sooner than 20 minutes (two-tailed one-sample Wilcoxon signed rank tests, Birds 6: $W = 1136$, $P < 0.001$; Bird 7: $W = 573$, $P < 0.001$).

By visiting some patches sooner than others, some patches were visited more often than others. Half of the birds (4, 6, 7 and 8), however, almost always visited three patches in the same bout so that the number of visits to each patch did not differ significantly (Bird 4: $X^2_2 = 2.87$, $P = 0.23$; Bird 6: $X^2_2 = 1.83$, $P = 0.4$ and Bird 7: $X^2_2 = 2.27$, $P = 0.32$ Bird 8: $X^2_2 = 0.4$, $P = 0.81$). This is not surprising for Birds 6 and 7 because the inter-visit interval

also suggested that those birds often visited all three patches on a single bout. For Birds 4 and 8, the number of visits to the three patches was not significantly different because these two birds too, often visited three patches per bout.

Did the hummingbirds visit the patches in sequence?

Overall, hummingbirds visited one patch during 50.9 % of the bouts, two patches 35.5 % and all three patches during only 13.4 % of the bouts. Hummingbirds revisited the same patch during a single bout on only eight occasions. For more than half of all the bouts (54.34 ± 2.84 % mean \pm S.E., $n = 8$), the hummingbirds revisited a patch that they had visited on the previous bout.

Furthermore, the birds visited one of the patches more often, visiting the “preferred” patch before visiting the other patches. For six of the eight birds the distribution of first visits to each patch was significantly different than expected by chance (Bird 1: $X^2_2 = 37.30$, $P < 0.001$; Bird 2: $X^2_2 = 30.65$, $P < 0.001$; Bird 3: $X^2_2 = 38.23$, $P < 0.001$; Bird 5: $X^2_2 = 74.95$, $P < 0.001$; Bird 7: $X^2_2 = 19.33$, $P < 0.001$ and Bird 8: $X^2_2 = 9.91$, $P < 0.01$). These birds visited one of the patches first in most of the bouts. Two birds however, made a similar number of first visits to each of the three patches (Bird 4: $X^2_2 = 0.97$, $P = 0.6142$ and Bird 6: $X^2_2 = 5.47$, $P = 0.06$).

The birds did showed a preferred sequence, since all the transition matrices based on the observed movements between the patches were significantly different from the expected matrices (Markovian chain Likelihood ratio test comparing observed and expected matrices $P < 0.001$, Table 1). Furthermore, out of the nine possible transitions between patches, including transitions within the same patch, all birds made one or more transitions significantly more often than expected by chance (transitions that had a Z-score > 1.96 were statistically different at a significant level of 0.05, Table 1; Figure 3).

Did the birds avoid revisiting empty flowers?

Overall the mean chance level across birds and all bouts was $48\% \pm 0.5$ (mean \pm S.E. $n = 1560$ bouts). This means that on average almost half of the flowers were rewarded at any one time. On average, the hummingbirds visited a rewarded flower first more often than the mean chance level of 48%. Since the probability of visiting a rewarded flower varied

across birds however, instead of using the average probability to determine whether individual birds visited a rewarded flower more often than the mean chance for all birds, we used a bird's own average proportion of rewarded flowers as the probability of visiting a rewarded flower for that bird. All birds performed significantly better than chance during at least one block of trials and no birds ever performed significantly worse than chance. (Figure 4; Table 2). The percentage of first visits to a rewarded flower did not change with experience (Wilcoxon matched-pairs test: $W = 17$, $P = 0.94$, $n = 8$) and the mean performance compared to their own mean chance of visiting a rewarded flower for all birds was greater than that expected by chance (Sign Test: $V = 36$, $P = 0.013$, $n = 8$). All birds visited at least 2 of the flowers significantly later or significantly equal to a 20 minute interval (two-tailed, one-sample Wilcoxon signed rank tests: Supplementary Material 2). This, combined with the bird's avoidance of empty flowers suggest that the hummingbirds were visiting and alternating between the order in which they visited the flowers and the patches. For instance, a hummingbird could visit in a first bout patches "A" and "B", during the second bout visit patch "C" first and then patches "A" and "B", on a third bout the bird would start again with the flowers in patches "A" and "B" and skip patch "C" entirely. This would also explain why some patches were visited more frequently than the others.

Discussion

When hummingbirds were presented with a flower array containing spatial structure (3 patches of 4 flowers) and temporal structure (20-minute flower refill delay), they adopted efficient spatio-temporal foraging patterns. Despite an interval of 10 minutes between foraging bouts, return intervals for each patch were significantly greater than 10 minutes, indicating non-random pattern in patch use (Figure 2). Markovian chain analysis revealed that each bird developed repeatable sequences of patch use both within and between foraging bouts (Figure 3). Efficient foraging was achieved through both patch sequences and avoidance of empty flowers within each patch (Figure 4). We argue that the sequences observed in hummingbird foraging are a form of traplining that marries spatial and temporal outcomes.

As expected, hummingbirds fed from the array every 10 minutes but fed from different patches at different intervals. Most of the hummingbirds in this experiment (6 out

of 8 birds) avoided visiting at least one of the three patches until after the 20-minute interval had passed, while visiting the other two patches every 10 minutes. Instead of visiting one patch per bout as we expected, most birds visited two patches in every bout and the third patch every other bout. This suggests that the birds matched their patch visits to the refill schedule of at least one of the patches and then visited in a repeated order, rather than learning the refill interval of the other two patches. Although hummingbirds can learn to time their visits to flowers that refilled at either 10 or 20-minute intervals (Henderson *et al.* 2006), here they used ordinality (i.e. a sequence), visiting two of the patches in the same order during most bouts and then visiting the third patch, only after they had found one of the two preferred patches to be unrewarded. In such a way the birds' repetitive movements in space produced regular patterns in time (Williams & Thomson 1998).

The use of ordinality to forage efficiently was also seen when rufous hummingbirds learned to feed from one flower in a patch at a specific location in the morning and another flower on a different patch at another location during the afternoon (Marshall *et al.* 2013). In that experiment, the birds appeared to have used the time of day coupled with the sequence in which the flower in each patch was rewarded. They can also do this at the level of patches of flower (Tello-Ramos *et al.* 2015). In this experiment too, hummingbirds seem to have learned both the refill interval of individual flowers and the order of patch visitation to correctly avoid empty flowers.

Territorial rufous hummingbirds used traplines to visit the three patches in a particular order. Regardless of the number of patches they visited in a bout, the hummingbirds began most of their foraging bouts by first visiting one particular patch and, depending on whether that particular patch was rewarded or not, continuing to their second preferred patch. This kind of step-wise sequence of visiting meant that the birds visited one particular patch more often than the other patches. By beginning most bouts at the same patch birds established the starting point of a trapline: the hummingbirds repeated the order in which they moved from one patch to another transitioning, for example, from patch "A" to patch "B" more often than from patch "A" to patch "C". Furthermore, this order in which hummingbirds moved between the three patches was followed within and between bouts. As traplining is defined as a behavior in which animals repeat the order in which they visit locations and not by the number of locations to visit or the distance between those

locations, even a short sequence of three patches can be considered a trapline. The concept of traplining (repeatable sequence) both connects spatial structure with timing, and simplifies timing by changing it from precise intervals to more simple ordinal information such that completion of a sufficiently long sequence exceeds the renewal interval of flowers when the sequence begins again. When hummingbirds repeatedly fed from a board that had artificial flowers presented one by one from 2 flowers to 3, 4 and then 5 flowers, the birds repeated the order in which they visited the flowers. In that experiment the hummingbirds flew the shortest possible routes around the flowers, even though the flowers were only 18 cm apart (Tello-Ramos *et al.* 2015).

Traplining is typically considered to be a foraging method used by nonterritorial hummingbirds that travel long distances between resources (Feinsinger 1976; Gill 1988; Tiebout III 1991; Garrison & Gass 1999; Temeles *et al.* 2006), based on changes in the visitation rates to feeders or to natural flowers (Garrison & Gass 1999; Temeles *et al.* 2006). But reports of territorial rufous hummingbirds feeding from flower on the edges of their territory in the early part of the day and working their way into flowers in the central parts of their territory can also be considered to be traplining (Gass & Montgomerie 1981; Paton & Carpenter 1984). Here we also confirm that this territorial hummingbird species will visit several patches of flowers in a sequential manner, which we consider also to be consistent with the description of traplining.

Traplining may help animals to outcompete intruders because resources are depleted before the intruders gain access to them (competition by depletion). This may explain why the hummingbirds visited more than one patch per bout, since by doing so they reduced the apparent standing crop available to intruders to a greater degree than if they had visited just one patch per bout: the birds drank as much as 200 μ l per bout, even though it is more typical for a territorial male rufous hummingbird to drink around half as much per bout (100 μ l of sucrose: Hurly 2003; Karasov *et al.* 1986). Although the crop size of these hummingbirds is about 600 μ l (Hainsworth & Wolf 1972), territorial males often drink much less when displaying to females and chasing off intruders from their territory (Carpenter *et al.* 1991) during the mating season. For our experimental birds, however, depleting the available resources may have been worth the cost of ingesting more sucrose per visit. Although we did not measure the frequency of intruders or how often the birds

revisited a patch after they had chased away an intruder, modifying the value of a male's territory does change his behavior: when the concentration of the sucrose in their feeder was increased from 14 % sucrose solution to 25 % territorial males fed less often and decreased the size of their territory, perhaps in response to an increase in intrusion rate caused by the increased value of the contents of their feeder (Bacon *et al.* 2011).

Taken together, by repeating the order in which they visited three patches whilst also avoiding flowers visited during the previous bout, hummingbirds were able to match the refill rate of individual flowers and avoid empty flowers. As these birds learned flower refill rate as well as flower locations their trapline was more than a repeated sequence of patch visits.

References

- Bacon I, Hurly TA, Healy SD (2011). Hummingbirds choose not to rely on good taste: information use during foraging. *Behavioral Ecology* **22**, 471-77.
- Bené F (1941). Experiments on the color preferences of the black-chinned hummingbirds. *The Condor* **43**, 237-42.
- Blem CR, Blem LB, Felix J, van Gelder J (2000). Rufous hummingbird sucrose preference: precision of selection varies with concentration. *The Condor* **102**, 235-38.
- Carpenter FL, Hixon MA, Hunt A, Russell RW (1991). Why hummingbirds have such large crops. *Evolutionary Ecology* **5**, 405-14.
- Castellanos MC, Wilson P, Thomson JD (2002). Dynamic nectar replenishment in flowers of *Penstemon* (*Scrophulariaceae*). *American Journal of Botany* **89**, 111-8.
- Dukas R, Waser NM (1994). Categorization of food types enhances foraging performance of bumblebees. *Animal Behaviour* **48**, 1001-06.
- Flores-Abreu IN, Hurly TA, Healy SD (2012). One-trial spatial learning: wild hummingbirds relocate a reward after a single visit. *Animal Cognition* **15**, 631-7.
- Freeman RB (1968). Charles Darwin on the routes of male humble bees. *Bulletin of the British Museum* **3**, 177-89.
- Garrison JSE, Gass CL (1999). Response of a traplining hummingbird to changes in nectar availability. *Behavioral Ecology* **10**, 714-725.
- Gass CL, Garrison JSE (1999). Energy regulation by traplining hummingbirds. *Functional Ecology* **13**, 483-92.

- Gill FB (1988). Trapline foraging by hermit hummingbirds: Competition for an undefended, renewable resource. *Ecology* **69**, 1933–42.
- Hainsworth, FR, Wolf LL (1972). Crop volume, nectar concentration and hummingbird energetics. *Comparative Biochemistry and Physiology A* **42**, 359–66.
- Healy SD, Hurly TA (1995). Spatial memory in rufous hummingbirds (*Selasphorus rufus*): A field test. *Animal Learning & Behavior* **23**, 63–8.
- Healy SD, Hurly TA (1998). Rufous hummingbirds' (*Selasphorus rufus*) memory for flowers: Patterns or Actual Spatial Locations? *Journal of Experimental Psychology: Animal Behavior Processes* **24**, 396–404.
- Henderson J, Hurly TA, Bateson M, Healy SD (2006). Timing in free-living rufous hummingbirds, *Selasphorus rufus*. *Current Biology* **16**, 512–5.
- Henderson J, Hurly TA, Healy SD (2001). Rufous hummingbirds' memory for flower location. *Animal Behaviour* **61**, 981–6.
- Hurly TA (2003). The twin threshold model: risk-intermediate foraging by rufous hummingbirds, *Selasphorus rufus*. *Animal Behaviour* **66**, 751–61.
- Hurly TA (1996). Spatial memory in rufous hummingbirds: memory for rewarded and non-rewarded sites. *Animal Behaviour* **51**, 177–83.
- Hurly TA, Healy SD (1996). Memory for flowers in rufous hummingbirds: location or local visual cues? *Animal Behaviour* **51**, 1149–57.
- Janzen, D. H. (1971). Euglossine bees as long-distance pollinators of tropical plants. *Science* **171**, 203–5.
- Karasov WH, Phan D, Diamond JM, Carpenter FL (1986). Food passage and intestinal nutrient absorption in hummingbirds. *The Auk* **103**, 453–64.
- Kodric-Brown A, Brown JH (1978). Influence of economics, interspecific competition, and sexual dimorphism on territoriality of migrant rufous hummingbirds. *Ecology* **59**, 285–96.
- Lemke TO (1984). Foraging ecology of the long-nosed bat, *Glossophaga soricina*, with respect to resource availability. *Ecology* **65**, 538–48.
- Lihoreau M, Chittka L, Raine NE (2010). Travel optimization by foraging bumblebees through readjustments of traplines after discovery of new feeding locations. *The American Naturalist* **176**, 744–57.
- Lihoreau M, Chittka L, Raine NE, Kudo G (2011). Trade-off between travel distance and prioritization of high-reward sites in traplining bumblebees. *Functional Ecology* **25**, 1284–1292.

Lihoreau M, Raine NE, Reynolds AM, Stelzer RJ, Lim KS, Smith AD, Osborne JL, Chittka L (2013). Unravelling the mechanisms of trapline foraging in bees. *Communicative & Integrative Biology* **6**, 1–4.

Lunau K, Papiorek S, Eltz T, Sazima M (2011). Avoidance of achromatic colours by bees provides a private niche for hummingbirds. *Journal of Experimental Biology* **214**, 1607–12.

Marshall RES, Hurly TA, Sturgeon J, Shuker DM, Healy SD (2013). What, where and when: deconstructing memory. *Proceedings of the Royal Society B* **280**, 20132194.

Marshall RES, Hurly TA, Healy SD (2012). Do a flower's features help hummingbirds to learn its contents and refill rate? *Animal Behaviour* **83**, 1163–69.

McDade LA, Weeks JA (2004) Nectar in hummingbird-pollinated neotropical plants II: Interaction with flower visitors. *Biotropica* **36**, 216–30.

Mistlberger RE, de Groot MHM, Bossert JM, Marchant EG (1996). Discrimination in circadian phase in intact and suprachiasmatic nuclei-ablated rats. *Brain Research* **739**, 12–8.

Noser R, Byrne RW (2010). How do wild baboons (*Papio ursinus*) plan their routes? Travel among multiple high-quality food sources with inter-group competition. *Animal Cognition* **13**, 145–55.

Ohashi K, Thomson JD (2009). Trapline foraging by pollinators: its ontogeny, economics and possible consequences for plants. *Annals of Botany* **103**, 1365–78.

Ohashi K, Thomson JD (2005). Efficient harvesting of renewing resources. *Behavioral Ecology* **16**, 592–605.

Paton DC, Carpenter FL (1984). Peripheral foraging by territorial rufous hummingbirds: Defense by exploitation. *Ecology* **65**, 1808–19.

Pizzo M, Crystal J (2002). Representation of time in time-place learning. *Animal Learning and Behavior* **30**, 387–93.

Possingham HP (1989). The distribution and abundance of resources encountered by a forager. *American Naturalist* **133**, 42–60.

R Development Core Team. 2012. R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria. ISBN 3-900051-07-0, URL <http://www.R-project.org>.

Riotte-Lambert L, Benhamou S, Chamaillé-Jammes S (2016). From randomness to traplining: a framework for the study of routine movement behavior. *Behavioral Ecology* **28**, 280–7.

Sandlin E (a) (2000). Cue use affects resource subdivision among three coexisting hummingbird species. *Behavioral Ecology* **11**, 550–59.

Sandlin E (b) (2000). Foraging information affects the nature of competitive interactions. *Oikos* **91**, 18–28.

Samuels M, Hurly TA, Healy SD (2014). Colour cues facilitate learning flower refill schedules in wild hummingbirds. *Behavioural Processes* **109**, 157–63.

Tello-Ramos MC, Hurly TA, Healy SD (2014). Female hummingbirds do not relocate rewards using colour cues. *Animal Behaviour* **93**, 129–33

Tello-Ramos MC, Hurly TA, Healy SD (2015). Traplining in hummingbirds: flying short-distance sequences among several locations. *Behavioral Ecology* **26**, 812–19.

Tello-Ramos MC, Hurly TA, Higgott C, Healy SD (2015). Time-place learning in wild, free-living hummingbirds. *Animal Behaviour* **104**, 123–9.

Thomson JD, Slatkin, M, Thomson BA (1997). Trapline foraging by bumble bees: II. Definition and detection from sequence data. *Behavioral Ecology* **8**, 199–210.

Williams NM, Thomson JD (1998). Trapline foraging by bumble bees: III. Temporal patterns of visitation and foraging success at single plants. *Behavioral Ecology* **9**, 612–21.

SUPPLEMENTARY MATERIALS

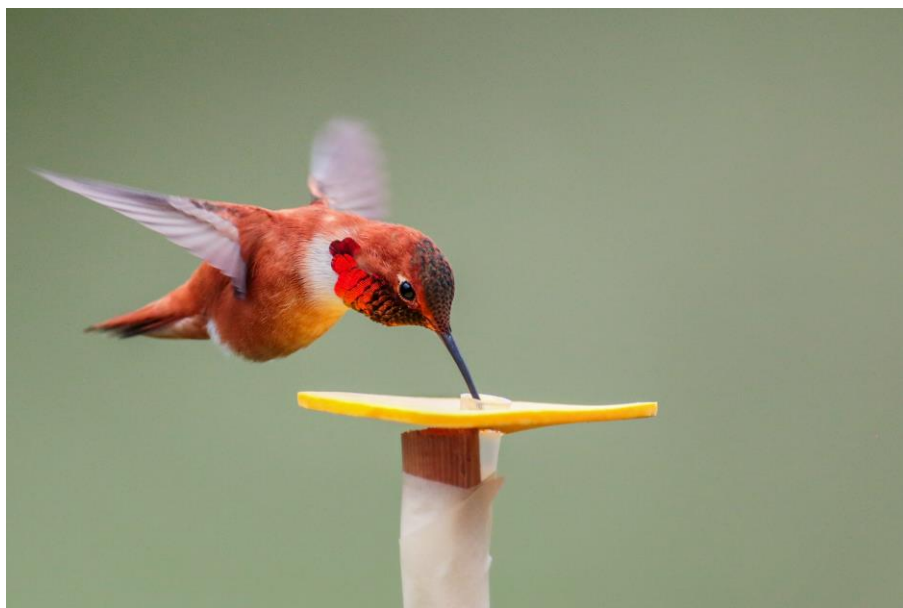


Figure S1 Photograph of a male rufous hummingbird feed from an artificial flower made of a syringe tip surrounded by a colored cardboard circle of 6 cm in diameter mounted on a 60 cm wooden stick. Photograph taken by Andrew T. Hurly

Table S1 Table showing the Median of the interval time the birds took to visit each of the 12 flowers, the number of times each flower was visited, the W statistic calculated from a two-tailed one-sample Wilcoxon signed rank tests with $\mu = 20$ and the P value of that test. Bold numbers are intervals that were either significantly greater or not significantly different to 20 minutes.

Bird	Patch	Flower	Median	n	W	P
1	A	1	15	84	721.5	< 0.001
		2	15	83	943	= 0.0011
		3	18	81	1078	= 0.02
		4	18	76	1212	= 0.44
	B	1	23	60	1175.5	= 0.05
		2	24	41	638.5	= 0.007
		3	25	48	924.5	< 0.001
		4	23	53	985	= 0.007
	C	1	23	51	869.5	= 0.01
		2	21.5	58	891	= 0.31
		3	21	46	588	= 0.16
		4	24	47	821	= 0.002
2	A	1	11.5	90	651.5	< 0.001
		2	11	100	651.5	< 0.001
		3	11	95	667.5	< 0.001
		4	12.5	84	625	< 0.001
	B	1	23	42	457	= 0.73
		2	23	43	482	= 0.50
		3	21	51	726	= 0.55
		4	22	57	763.5	= 0.86
	C	1	12	90	435.5	< 0.001
		2	13	90	541	< 0.001
		3	13	83	560	< 0.001
		4	15	82	541	< 0.001
3	A	1	12	103	594	< 0.001
		2	14	100	684	< 0.001
		3	14	102	703.5	< 0.001
		4	13	106	563.5	< 0.001
	B	1	11	110	612	< 0.001
		2	11	104	570	< 0.001
		3	14	101	690	< 0.001
		4	11.5	108	534.5	< 0.001
	C	1	19.5	76	984	= 0.092
		2	20	69	948	= 0.54
		3	20	69	926.5	= 0.44
		4	20	74	968.5	= 0.15

4	A	1	13	101	831.5	< 0.001
		2	15	94	943.5	< 0.001
		3	15	94	1037	< 0.001
		4	13	97	1029.5	< 0.001
	B	1	15	92	1194.5	< 0.001
		2	15.5	86	1141.5	= 0.004
		3	15.5	88	1093.5	< 0.001
		4	15	93	1107	< 0.001
	C	1	20	73	1115.5	= 0.58
		2	20	73	1333	= 0.59
		3	20.5	76	1298	= 0.93
		4	22	71	1174	= 0.82
5	A	1	22	49	687.5	= 0.45
		2	22	55	846.5	= 0.52
		3	22	53	748.5	= 0.77
		4	22	43	502.5	= 0.72
	B	1	12	86	729.5	< 0.001
		2	12	84	766	< 0.001
		3	12	84	951	< 0.001
		4	13	85	991.5	< 0.001
	C	1	12	103	997	< 0.001
		2	12	99	1087	< 0.001
		3	13	91	846	< 0.001
		4	13	100	952.5	< 0.001
6	A	1	17	88	1261	= 0.019
		2	14.5	94	959.5	< 0.001
		3	18	78	943.5	= 0.02
		4	18.5	80	1251.5	= 0.20
	B	1	15	85	1013.5	= 0.001
		2	15	88	1155	= 0.001
		3	17	85	1141	= 0.014
		4	19	74	1054.5	= 0.20
	C	1	15	100	1172.5	< 0.001
		2	13	109	1088	< 0.001
		3	17	98	1444.5	= 0.001
		4	18	95	1437.5	= 0.009
7	A	1	19	79	1225.5	= 0.16
		2	16	80	838	= 0.001
		3	19.5	56	614.5	= 0.27
		4	21	64	843	= 0.463
	B	1	15	104	756.5	< 0.001
		2	15	95	776.5	< 0.001
		3	15	95	791.5	< 0.001
		4	15	86	684	< 0.001
	C	1	15.5	88	1006.5	< 0.001
		2	14	99	690	< 0.001

8		3	15	95	849	< 0.001
		4	15.5	80	796	< 0.001
	A	1	14	92	1229.5	= 0.001
		2	14	90	1117.5	= 0.002
		3	17	87	1441	= 0.17
		4	17	87	1660.5	= 0.11
	B	1	17	86	1578.5	= 0.27
		2	20.5	88	1829.5	= 0.86
		3	19.5	76	1251.5	= 0.46
		4	18	82	1361.5	= 0.28
	C	1	17	97	1775.5	= 0.06
		2	17.5	96	1660	= 0.04
		3	18	83	1394.5	= 0.36
		4	17.5	92	1606	= 0.10

Table 1 Table with the G^2 statistic from Markovian chain Likelihood ratio test comparing observed and expected matrices of transitions between patches within and between bouts. Transitions that had a Z-score > 1.96 were statistically different at a significance level of 0.05. Bold numbers show transitions between patches that were used more often than expected by chance.

Bird	G ²	P value	Z score for each transition		
1	48.23	< 0.001	Green	Green	Pink
				-5.11	2.66
			Purple	2.09	-3.69
2	51.13	< 0.001	Pink	3.50	0.81
					-4.65
			Blue	Green	Pink
3	62.08	< 0.001		-4.04	-1.85
			Blue	3.47	-2.85
			Green	0.95	4.43
4	90.44	< 0.001	Pink		-4.94
			Blue	Purple	Pink
5	114.65	< 0.001		-5.21	1.61
			Purple	2.92	-4.92
			Pink	2.60	2.81
6	70.94	< 0.001			-5.13
			Green	Purple	Orange
				-6.64	1.79
7	81.59	< 0.001	Green	3.43	-5.15
			Purple	3.43	3.08
			Orange		-6.36
8	90.03	< 0.001		Blue	Orange
			Blue	-5.48	5.04
			Orange	-2.27	-5.16
9	90.03	< 0.001	Pink	6.92	0.71
					-6.66
			Blue	Green	Orange
10	90.03	< 0.001		-5.15	1.52
			Blue	3.88	-5.81
			Green	1.22	4.14
11	90.03	< 0.001	Orange		-5.18
			Blue	Purple	Pink
12	90.03	< 0.001		-6.02	3.21
			Purple	2.78	-5.83
			Pink	2.97	2.70
13	90.03	< 0.001			-5.48
			Blue	Orange	Pink
				-4.18	-1.40
14	90.03	< 0.001	Blue	3.38	-5.36
			Orange	0.87	6.64
			Pink		-7.41

Table 2 Table showing the mean probability for each bird of visiting a rewarded flower, Z score statistic calculated from a Binomial test and the P value of that test divided in to bins of 50 bouts. Bold numbers are significantly different from chance.

	Mean chance	1- 50 bouts		51-100 bouts		101-150 bouts		151-200 bouts	
		<i>Z</i>	<i>P</i>	<i>Z</i>	<i>P</i>	<i>Z</i>	<i>P</i>	<i>Z</i>	<i>P</i>
Bird 1	0.54	2.7	0.005	-0.14	0.89	2.7	0.005	2.15	0.028
Bird 2	0.45	1.14	0.25	1.71	0.088	0	0.99	1.99	0.047
Bird 3	0.4	3.03	0.002	1.01	0.3123	2.17	0.032	1.01	0.312
Bird 4	0.44	3.28	0.001	1.85	0.064	4.13	< 0.0001	2.86	0.004
Bird 5	0.51	1.7	0.088	0	0.99	0.85	0.39	2.44	0.013
Bird 6	0.5	-0.42	0.67	1.56	0.118	2.4	0.015	2.12	0.032
Bird 7	0.44	-0.41	0.88	2.14	0.033	3.56	0.0003	2.86	0.004
Bird 8	0.55	-0.28	0.77	2.56	0.0089	1.99	0.043	1.14	0.25

Figure legends

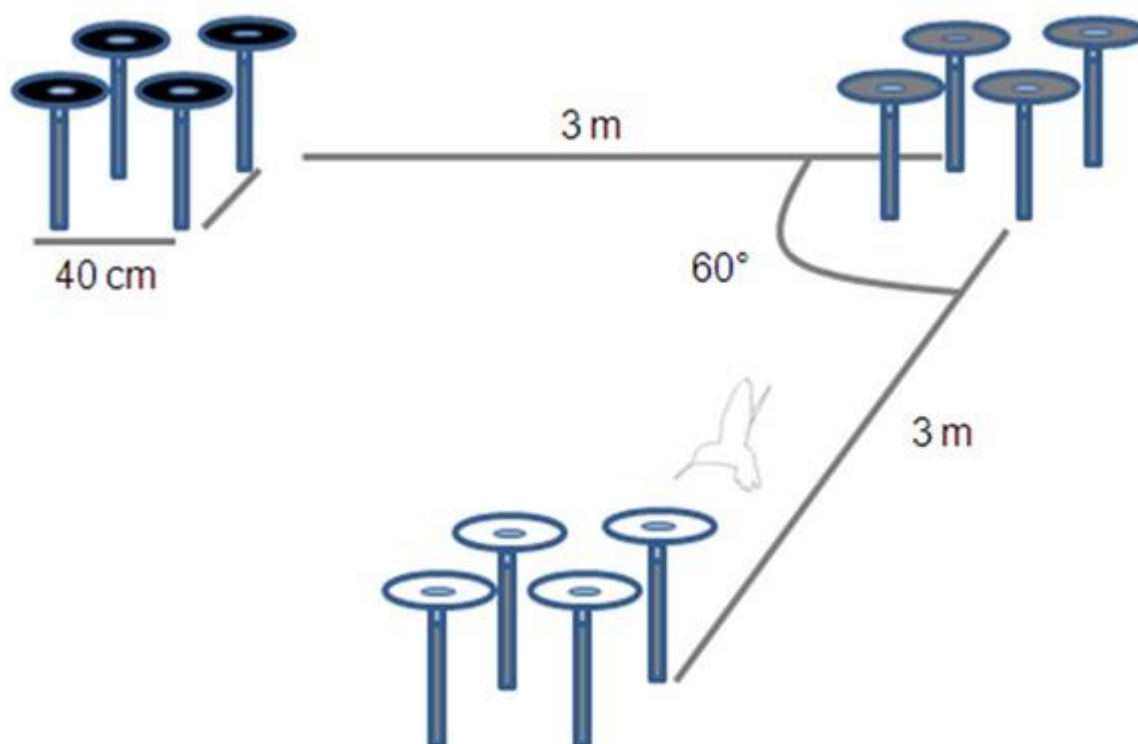


Figure 1 Diagram of the three-patch flower array. Patches of flowers were separated by 3 m and flowers within patches were 40 cm apart. Birds fed freely from the artificial flowers but these were refilled (25 μ l of 25 % sucrose solution) only after 20 minutes had passed from the last visit.

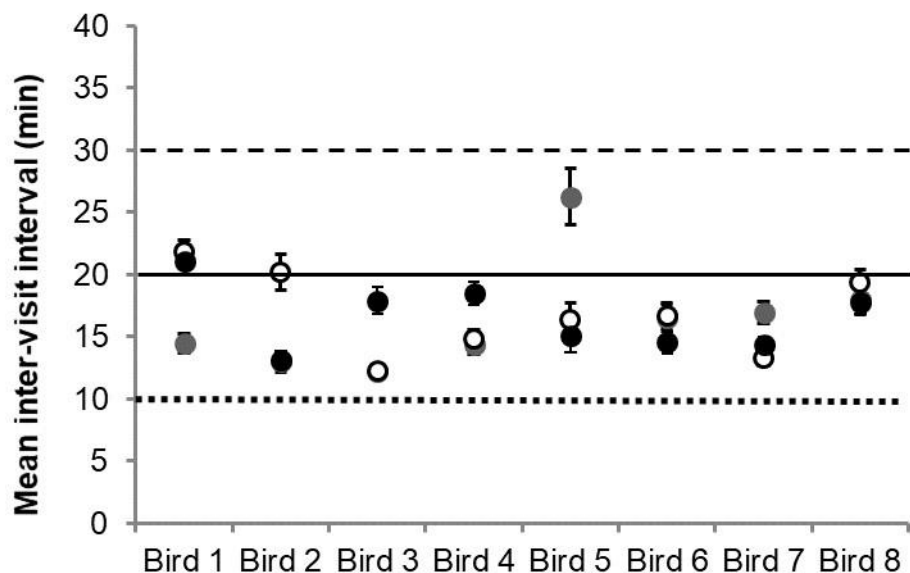


Figure 2 Mean (\pm SE, $n = 8$) of the inter-visit interval time in minutes each bird took to revisit each patch. White, black and gray circles represent different patches. The dash line at 30 minutes represents the interval that would be expected if hummingbirds visited the patches in the same order at the appropriate time. The solid line at 20 minutes represents the interval at which patches were replenished. The dotted line at 10 minutes represents the interval at which hummingbirds regularly feed.

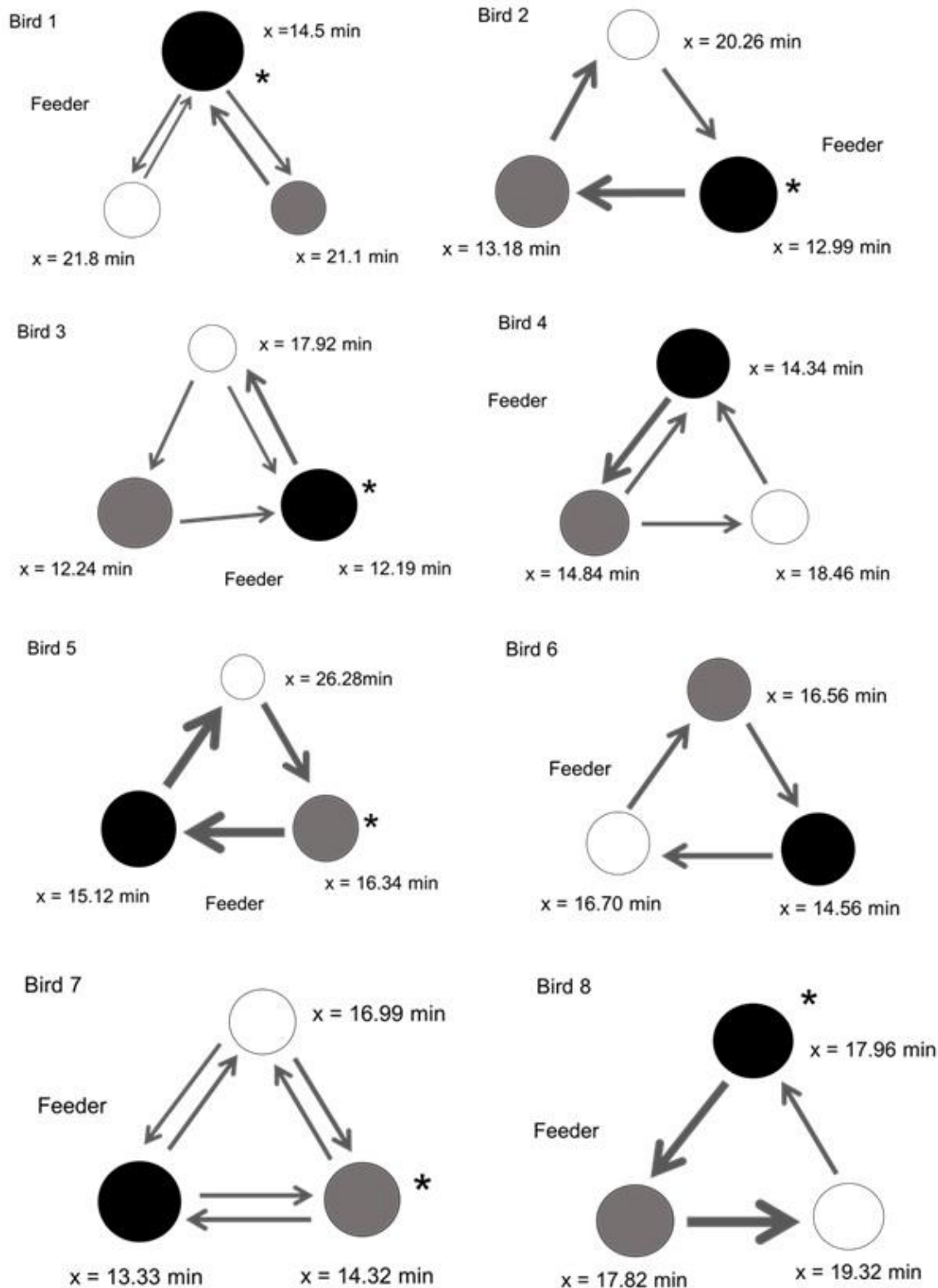


Figure 3 Diagrams of the transitions between patches that occurred significantly more often than expected by chance for each of the eight birds. The different colored circles represent the three different patches each bird could visit. The circle size is proportional to the frequency of visits to that patch and is comparable within and between birds. Arrow size is proportional to the conditional probability of the transition. Diagrams only include the transitions with significant Z- scores ($Z > 1.96$). The asterisk denotes the patch that was visited first in a bout most frequently. x denotes the mean inter-visit interval for that patch.

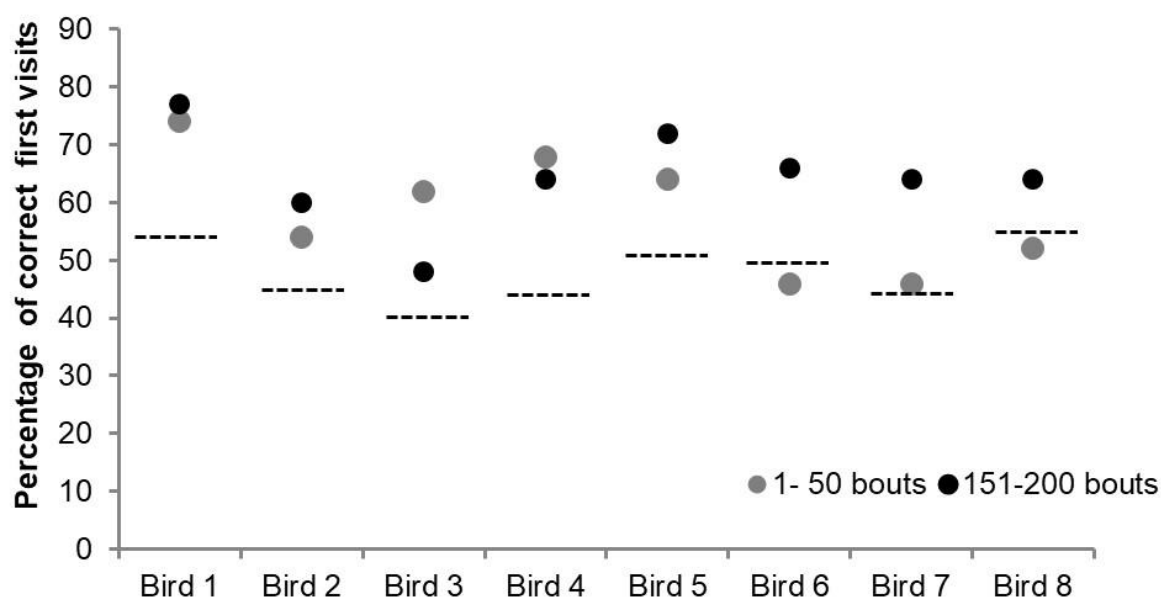


Figure 4 Percentage of correct first visits made during the first and last fifty bouts (different symbols; grey circles 1 to 50 and black circles 151 to 200 bouts). The dashed lines represent the mean chance for each bird calculated as the mean proportion of flowers containing reward during each bout.